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(54) Title: CULTURED PROTEIN HYDROLYSATE

(57) Abstract: This invention relates to a process for the production of a cultured savory base which comprises hydrolysing a protein-containing material using a combination of at least one enzyme with at least one thermotolerant lactic acid bacteria strain selected for its ability to provide a glutaminase activity. The invention also relates to an isolated strain of thermotolerant lactic acid bacteria strains selected for their ability to provide a glutaminase activity and their use for preparing seasonings, flavour and culinary and petfood products.

Cultured protein hydrolysate

.In US patent No 5,965,178, Baensch et al. disclose a process for the production of a seasoning which comprises preparing a fermented koji from protein-containing and carbohydrate materials and then hydrolysing the koji material in the presence of a culture of lactic acid bacteria. The seasoning composition is obtained without preparing a moromi.

Further Biological plant protein hydrolysates to be used as neutral body givers, as bases for processed flavours, in culinary applications like bouillons, soups and sauces, are also developed as well as for liquid seasonings

The problem is that biological hydrolysates are generally more expensive to produce than HPP due to lower yields, higher equipment costs and higher raw material prices e.g. enzymes (commercial or own production by fermentation).

Finally, it is also known to hydrolyse wheat gluten enzymatically using commercially available enzymes. The critical points in the development of wheat gluten hydrolysate (with food approved enzymes) processes are mainly the high costs of these enzymes, separation of solids from the hydrolysates with high yield and microbiological protection of the hydrolysates since they are run without or low salt concentrations, at temperatures permissible for certain spoilage micro-organisms.

The present invention aims to provide a natural and "soft technology" procedure to prepare seasonings, which is very much desired by consumers these days.

Summary of the invention

Surprisingly, it has been found that some lactic acid bacteria strains used in combination with enzymes may have a synergistic activity in producing savory bases which are good body givers and contain significant amounts of "in process produced" MSG (Mono Sodium Glutamate) OR GLUTAMIC ACID. In the present specification, MSG relates either to Mono Sodium Glutamate or glutamic acid.

Accordingly, this invention provides a process for the production of a cultured savory base which comprises hydrolysing a protein-containing material using a combination of at least one enzyme with at least one thermotolerant lactic acid bacteria strain selected for its ability to provide a glutaminase activity.

In a preferred embodiment, the enzyme is any food approved technical endo- or exo- peptidase and/or protease, deaminase, transaminase or amyloglucosidase, for example.

In a preferred embodiment, the lactic acid bacteria is used in the form of a starter (innoculum). The lactic bacteria may be selected from the group consisting of facultative heterofermentative Lactobacilli, *Lactobacillus rhamnosus*, *Lactobacillus delbrucki*, *Lactobacillus casei*, *Lactobacillus paracasei*, *Lactobacillus sp.*

In a most preferred embodiment the lactic acid bacteria strain is *Lactobacillus rhamnosus* (NCC 858) (CNCM I-2433).

The ratio of Enzyme : Lactic acid bacteria being preferably of about 1:1 to about 4:1 based on %(w/w) addition of the ingredients.

The hydrolysis may be carried out for a time sufficient for the lactic acid bacteria to produce 1 to 4 % MSG OR GLUTAMIC ACID in the hydrolysate or to have a DH of at least 20%. The hydrolysis of the protein-containing material is thus preferably carried out at a temperature of at least about 30-50°C. The pH is preferably maintained between about 5 to 6. The reaction may be carried out for at least 12 hours.

The hydrolysate may be further processed downstream by means of thermal inactivation, filtration and / or centrifugation to produce a raw sauce. In a preferred embodiment, the hydrolysate is pasteurized, salt is added and the hydrolysate is filtered to separate solids from the liquid phase.

The process according to the present invention has numerous advantages. It efficiently competes spoilage micro-organisms, like coliform bacteria. It is entirely biological and good hydrolysis of the protein material is obtained.

Moreover, it is shorter than any process for biological hydrolysates described up to date.

Furthermore the above mentioned process employs synergistic effects of added enzymes (proteases, peptidases) and enzymes provided by the lactic acid bacteria. Compared to similar hydrolysates, produced by enzymes only, the cultured hydrolysates show an increased degree of hydrolysis and an increased yield of mono sodium glutamate (MSG).

Another main object of the present invention relates to an isolated thermotolerant lactic acid bacteria (LAB) strain selected for its ability to provide a glutaminase activity. The selected strain can eliminate reducing sugars such as glucose and maltose. The LAB strain may also provide increased release of proline and MSG or GLUTAMIC ACID from peptide bonds.

In a preferred embodiment the lactic acid bacteria strain is selected from the group consisting of facultative heterofermentative Lactobacilli, *Lactobacillus rhamnosus, Lactobacillus delbrucki, Lactobacillus casei, Lactobacillus paracasei, Lactobacillus sp.*

In a most preferred embodiment the lactic acid bacteria strain is *Lactobacillus rhamnosus* (NCC 858) (CNCM I-2433).

In a last aspect, the invention relates to the use of thermotolerant lactic acid bacteria (LAB) strains having the above traits as process micro-organisms for the preparation of seasonings.

The strains according to the present invention provide a microbiological protection of the process and a significant elimination of the reducing sugars leading to decreased uncontrolled Maillard reactions, which improves the shelf-live stability and applicability of the product.

Moreover, the selected strains do not impair the body-giving and taste characteristics of the hydrolysate and even contribute to an improved body and taste (higher degree of hydrolysis, higher MSG or GLUTAMIC ACID yield).

Detailed description of the invention

Within the following description, the abbreviation cfu ("colony-forming-unit") designates the number of bacterial cells as revealed by microbiological counts on agar plates.

In the specification, all percentages are given on the basis of weight except where specifically stated otherwise.

As quality marker to monitor the efficiency of protein hydrolysis the degree of hydrolysis (DH) is formulated, it is : DH = ("conc. of free α-amino nitrogen x100)/ "conc. of total nitrogen"

Moreover, "NCC" designates Nestlé Culture Collection (Nestlé Research Center, Vers-chez-les-Blanc, Lausanne, Switzerland).

With respect to the first object of the present invention, the protein containing material is from plant or animal origin. Plant protein material may be wheat gluten, defatted soya-grits, defatted soya beans, corn gluten, rice gluten, sun flower presscake, for example. It is preferably wheat gluten. Animal protein material such as milk proteins, chicken meal, beef or pork, for example, may also be used as substrate for the present process. The protein containing material is preferably used in a dry, powdered form, or as grits.

The protein containing material is preferably mixed with water so as to obtain a porridge/slurry. The resulting slurry is then subjected to a hydrolysis by using a combination of at least one enzyme with at least one thermotolerant lactic acid bacteria strain selected for its ability to provide a glutaminase activity.

The enzyme according to the present invention may be any endo- or exo-peptidase, deaminase, transaminase, glutaminase or amyloglucosidase, for example. It is preferably used Flavorzyme®, Alcalase®, Dextrozyme®, AMG®. These enzymes were provided by NOVOZYMES NORDISK FERMENT Ltd. (Novo Nordisk Ferment AG, Dittigen, Switzerland). The enzyme may be used alone or in combination. The enzyme may be used in an amount which can vary according to the enzyme used. If a protease is used, the amount is preferably of at

least 1 % by weight based on wheat gluten and preferably from about 1% to about 4%. If a carbohydrase is used, the amount is preferably of about at least 0.01% by weight based wheat gluten .

At least one thermotolerant lactic acid bacteria (LAB) strain is used in combination with the enzyme. The lactic acid bacteria strain has the traits as further described. It may be selected from the group consisting of facultative heterofermentative Lactobacilli, *Lactobacillus delbrueckii, Lactobacillus casei, Lactobacillus paracasei* and *Lactobacillus rhamnosus* and is most preferably *Lactobacillus rhamnosus* (NCC 858) (CNCM I-2433).

The selected lactic acid bacteria strain is advantageously added in the form of a starter resulting in a titer of about 10^4 to about 10^7 cfu/g in the hydrolysate at the start of the reaction. The starter may be added to the hydrolysate reaction in an amount of at least 0.1 % (v/v) and preferably from about 0.5 to 2% (v/v) or so that the amount in the hydrolysate reaction at the start is preferably of about $5x10^5$ to about $2x10^6$ cfu/g.

The ratio enzyme: lactic acid bacteria is preferably of about 1:1 to about 4:1 based on % (w/w) addition of the ingredients.

In a preferred embodiment the present process is carried out by hydrolysing the protein containing material, by mixing 15 to 30 % of the protein containing material in 65 to 80% water to obtain a slurry, then adding to the slurry about 1.0% to about 4.0 % (based on wheat gluten) of at least one enzyme and about 0.1% to about 1% of a starter (an inoculum) of at least one lactic acid bacteria strain having a titer of about $10^9 - 10^{11}$ cfu/g (in the concentrated starter culture). The percentages are given by weight based on the total reaction.

The hydrolysis of the protein-containing material may be carried out at a temperature of at least 43°C, and preferably from about 45 to about 48°C. The pH may be of about 5 to 6. The pH can be maintained by adding acetate buffer pH or calcium carbonate, for example in the range between pH 5 - 6.

The hydrolysis is preferably carried out for a time sufficient for the lactic bacteria strain to grow by 2-3 log, or to create 1-4% mono-sodium glutamate or

glutamic acid in the hydrolysate, or to have a degree of hydrolysis (DH) of at least 20%.

In a preferred embodiment, the hydrolysis may be carried out for at least 12 hours, and most preferably from about 15 to about 24 hours.

During this protein hydrolysis reducing sugars are released, due to enzymatic side activities in the commercial enzyme preparations. The reducing sugars are reaction partners in Maillard reactions and can thus cause browning and the creation of bitter notes during storage. The selected lactic acid bacteria which is thermotolerant, eliminates glucose and maltose, but does not impair the function of the process-enzymes or produce off-flavours. Due to the elimination of the sugars, the lactic acid bacteria strain will also function as a protective culture and has antimicrobial activity against gram$^-$ and gram$^+$ bacteria. The obtained hydrolysate is then low in reducing sugars. Glucose can be eliminated completely during the hydrolysis, while the maltose concentration may be partly reduced.

Then, the hydrolysate may be treated in different ways in order to prepare a liquid, a paste or a powder as conventionally known.

In a preferred embodiment, the hydrolysate is dried (vacuum drying at 60-90°C at about 15 to 4 mbar for at least 3hours, or spray drying in Niro spray dryer at an inlet air temperature of 140°C – 180°C and outlet air temperature of about 90°C so as to obtain a powder with a dry matter content of at least 98%. The obtained savoury base may be used in process flavor applications, for example or alternatively the hydrolysate can be used in liquid seasoning.

In another embodiment, up to 15% NaCl can be added after the hydrolysis, and the hydrolysate is heated up to about 90°C and kept at this temperature for about 10 minutes to inactivate enzymes and microbial metabolic activity (pasteurization).

In another embodiment, the inactivated hydrolysate is cooled down and kept at about 55°C and then filtered either through plate and frame filters or alternatively by using a Hoesch filter press. After filtration a cleared raw sauce with a TS of 30 – 35% is obtained.

In order to stabilize the raw sauce even further, it can be reacted by incorporating cysteine in an amount of from 0.01 to 0.1 part by weight and then by heat treating the mixture at 95°C to 110°C for 1 to 5 hours, as described in US patent No. 5,480,663.

The cultured hydrolysis produces hydrolysates with a good round body (mouthfeel) and neutral taste with good body-giving potential and with a decreased content of reducing sugars.

Furthermore, low levels of reducing sugars lead to decreased uncontrolled Maillard reactions, which improves the shelf-live stability of the product.

Hydrolysates were also prepared by using enzymes only. It has been shown that such hydrolysates contain higher concentrations of reducing substances and lacks lactate, which is produced during cultured hydrolysis. The cultured hydrolysates according to the present invention show an increased degree of hydrolysis and an increased yield of MSG or GLUTAMIC ACID. The cultured protein hydrolysis process appears to be a selfregulating system, where the parameters enzyme concentration, inoculum dosage, metabolic activity of the micro-organism, pH of the reaction and temperature are in a dynamic equilibrium with each other.

The hydrolysates according to the present invention are good candidates for body givers in process flavours and culinary applications (see examples).

In a preferred embodiment, the cultured hydrolysate is used directly in its liquid or dried form so as to enhance or impart a savory type flavour in culinary products, for example. The amount of the liquid form is preferably of about 0.05 to 750 g per kg of culinary product or petfood product, depending on the required flavor intensity. The dried form of the flavouring agent can be used in an amount of 0.2-250 g per kg product.

According to another object of the present invention, several strains of thermotolerant lactic acid bacteria (LAB) were screened according to their properties and physical parameters: food grade and having GRAS status,

capability to metabolize both glucose and maltose, thermotolerance between 40°C and 55°C.

The selected LAB strains are food-grade. They are thermotolerant (i.e. growth between 40°C – 55°C) and growth on / isolation from plants. They also have the ability to metabolize glucose and maltose. They do not impair the function of the process-enzymes, the body-giving and taste characteristics of the hydrolysate and do not produce off-flavours.

In a preferred embodiment the LAB is selected from the group consisting of facultative heterofermentative Lactobacilli, *Lactobacillus rhamnosus, Lactobacillus delbrueckii, Lactobacillus casei, Lactobacillus paracasei and other Lactobacillus sp,* for example.

In a more preferred embodiment, the LAB is *Lactobacillus rhamnosus* NCC 858 (CNCM I-2433).

The *Lactobacillus rhamnosus* (NCC 858) strain was deposited by way of example under the Budapest Treaty at the Collection Nationale de Cultures de Microorganismes (CNCM), Institut Pasteur, 28 rue du Docteur Roux, 75724 Paris Cedex 15, France, on April 5, 2000, under the reference CNCM I-2433.

BIOCHEMICAL CHARACTERIZATION OF THE SELECTED STRAIN

***Lactobacillus rhamnosus* (NCC 858)**

- Gram positive microorganism, non-motile, non sporulating.
- Rod shaped cells; 0.8 – 1 μm by 2-4 μm, often with square ends, occur singly or in short chains.
-Microaerophilic micro-organism with facultative heterofermentative metabolism, production of L(+) lactic acid.
- Catalase negative, production of CO_2 facultative
- Fermentation of the sugars: Amygdaline (+), Arabinose (+/-), Cellobiose (+), Esculin (+), Gluconate (+), Mannitol (+), Melecitose (+), Melibiose (-), Raffinose (-), Ribose (+), Sorbitol (+), Sucrose (+), Xylose (+).

- Growth up to 48°C possible.

In another embodiment, the invention relates to the use of thermotolerant lactic acid bacteria (LAB) strains having the above traits as process micro-organisms for the preparation of seasonings. The selected LAB may be used in a process for preparing savory bases as described above, for example. Surprisingly it was found that culturing using *Lactobacillus rhamnosus* NCC 858, for example, could eliminate the use of technical glutaminase for conversion of glutamine to glutamate. Additionally NCC 858 also shows some proteolytic activity.

The LAB strains according to the present invention do not impair the body-giving and taste characteristics of the hydrolysate and even contribute to an improved body and taste (higher degree of hydrolysis, higher MSG OR GLUTAMIC ACID yield). The LAB strains may also provide a microbiological protection of the flavouring process and a significant elimination of the reducing sugars leading to decreased uncontrolled Maillard reactions, which improves the shelf-live stability of the product.

The following examples are given by way of illustration only and in no way should be construed as limiting the subject matter of the present application.

Examples

Example 1

Strain NCC 858 was used for the initial experiments to set up suitable reaction conditions. For the initial trials the pH of the reaction was maintained at 5.8 by dosing NaOH to the system. The reactions were based on the following recipe:

Ingredients	***% (w/w)*** based on total reaction
Wheat gluten	22
Water	76.25
Flavorzyme® (based wheat gluten)	1

Glutaminase C200® (based on wheat gluten)	0.1
Acetate buffer pH 5.8	0.25
LAB starter	5.10^5 cfu/g (0.4%)

Table 1 summarizes the results of the growth test with NCC 858, to find the optimal growth and reaction temperature in a cultured wheat gluten hydrolysate process.

Table 1:Growth test in cultured process medium

Temp[1].	Cfu/g[2] [0h]	Cfu/g[2] [16h]	RS[3] [0h]	RS[3] [16h]	DH[4] [16]
45°C	1.10^6	$1.5.10^9$	Glc 0.3% Mal 1%	Glc <0.05% Mal 0.3%	23-27%
55°C	1.10^6	3.10^2	Glc 0.3% Mal 1%	Glc 0.48% Mal 1.2%	23-26%

[1] Temperature of hydrolysis reaction, [2] indicated are the counts for NCC 858 in the hydrolysate. For coliforms, bacilli, spores the counts were <10 cfu/g after 16h, [3] reducing sugars, [4] degree of hydrolysis as ratio between free α-amino nitrogen and total nitrogen concentration.

The results clearly show (cfu and sugar concentrations) that at 45°C there is good growth of the micro-organism during the 16h of hydrolysis. Subsequent hydrolyses have though shown that temperatures up to 47°C are tolerated by strain NCC 858. Monitoring of the degree of hydrolysis shows that the efficiency of hydrolysis of the enzymes is as efficient at 45°C, as it is at 55°C.

- **Determination of possible dosage range for NCC 858 starter in cultured wheat gluten hydrolysate process**

It has been shown, that a dosage range for the inoculi between 1.10^4 cfu/g and 1.10^6 cfu/g does not have a negative influence on the hydrolysis.

Microbiological monitoring for coliforms, AMC and AMS showed that there was good protection exerted by NCC 858 in the entire dosage range.

Dosages lower than 1.10^4 cfu/g were not tried, in these cases the inoculum can no more dominate the contaminating flora present in the raw material.

- **Cultured hydrolysis process with Flavorzyme®/Alcalase® as only enzymes**

The dosage range of 1 – 3% Flavorzyme® was tested in this series. Alcalase® was added to 0.1% in all trials. The recipe for the hydrolysates is otherwise identical to the one already described above.

Table 2 shows the comparison of the analytical values for the above quoted trials, the enzyme versions of wheat gluten hydrolysate. As reference values 2% Flavorzyme®, 0.1% Alcalase® and 4% Flavorzyme®, 1% GlutaminaseC200®, 0.1% Alcalase®) were used.

Table 2: Comparison of analytical results for the process using Flavorzyme® / Alcalase® as only enzymes at different dosages.

	Dosage of Inoculum cfu/g	Enzyme conc.	MSG[5]	DH[1]	Glc[3]/ Mal[4]	RS[2]	DM
			[% w/w]				
Non cultured hydrolysate	No	2% Flav, 0.1% Alc	0.27	24.55	n.a. / n.a.	1.83	32.9
Cultured hydrolysate	2.10^5	2% Flav, 0.1% Alc	2.67	32.71	0.05 /0.72	1.02	32.3
Cultured hydrolysate	5.10^5-1.10^6	2% Flav, 0.1% Alc	2.49	30.6	0 / 0.67	0.93	32.3
Cultured hydrolysate	2.10^6	1% Flav, 0.1% Alc	1.34	22.96	0.05 / 0.69	0.93	31.4
Cultured hydrolysate	2.10^6	3% Flav 0.1% Alc	2.9	33.04	0 / 0.58	1.05	33.4
Non cultured hydrolysate	No	4% Flav, 1% Gln, 0.1%Alc	3.2	35.7	n.a. / n.a.	2.24	29.7

[1] degree of hydrolysis [2] Reducing substances (sugars and others), [3] Glucose, [4] Maltose, [5] Mono sodium glutamate, Flav: Flavorzyme®, Gln: Glutaminase, Alc : Alcalase®.

The data in table 2 show clearly that hydrolysates made with only 2% Flavorzyme® and NCC 858 starter only, produce good levels of the quality markers MSG or GLUTAMIC ACID and DH. The data are comparable to process conditions where a hydrolysate was made with 2% Flavorzyme® and 0.75% glutaminase (Glutaminase C200®) and starter NCC858 (data not shown). In other words the addition of commercial glutaminase does not improve the MSG or GLUTAMIC ACID yield or the degree of hydrolysis in the cultured process.

The process using only 2% Flavorzyme and 0.1% Alcalase but no starter results in a significantly lower degree of hydrolysis and only base levels of MSG or glutamic acid.

Process conditions using 3% Flavorzyme, 0.1% Alcalase and NCC858 starter show comparable results to those were 4% Flavorzyme, 0.1% Alcalase and 1% Glutaminase C200 were used.
To summarize addition of starter NCC858 to the hydrolysis system helps to reduce the quantity of Flavorzyme® and allows to eliminate the commercial glutaminase from the production of cultured wheat gluten hydrolysate.

Example 2: preparation of a savoury base (or dried cultured hydrolysate)

In order to prepare a cultured wheat gluten hydrolysate, 22 % of wheat gluten is mixed in 75.65% of water to obtain a slurry. The obtained slurry is then hydrolyzed, by adding 1% Flavorzyme® (based on wheat gluten) and 0.1% of Alcalase® and 1% of a starter of *Lactobacillus rhamnosus* (NCC 858) (CNCM I-2433) having a titer of $5x10^5$ cfu/g.

The pH for the hydrolysis is maintained at about 5.8 by adding acetate buffer pH 5.8 to 0.25% (w/w) and the reaction is carried out at 45-48°C during 16 hours so as to obtain the cultured hydrolysate.

Then, the hydrolysate is vacuum dried at 66°C at about 15 to 4 mbar for ≥3 hours so as to obtain a powder. The powder has a dry matter content of at least 98%. This powder may be used as described in example 5.

Example 3 : preparation of a raw sauce (liquid savory base)

A cultured hydrolysate is prepared as described in example 2. After the hydrolysis 15% of NaCl is added. The hydrolysate is then heated up to about 90°C and kept at this temperature for about 10 minutes to inactivate enzymes and microbial metabolic activity (pasteurization).

The inactivated hydrolysate is cooled down and kept at about 55°C and then filtered by using a Hoesch filter press. After filtration a cleared raw sauce with a TS of 30 – 35% is obtained. This raw sauce may be utilized for the preparation of seasonings (cf example 4).

The raw sauce can also be evaporated and vacuum dried and then milled into a powder. This powder can then be used in culinary and flavor applications as demonstrated in examples 5 and 6.

Example 4 : liquid seasoning

In order to prepare a liquid seasoning, the following ingredients are mixed together in a stirred reactor :

Ingredient	**[%]**
Cultured wheat gluten hydrolysate with enzymes	50
Water	33.8
Salt	13
Acetic Acid	0.5
Lovage Flavor	0.1
MSG	2.0
Caramel Color	0.6

The mixture is then pasteurized at a temperature of 95°C during 15 minutes and, subsequently the seasoning is transferred into brown bottles for storage and application tests.

Example 5 : Application in a culinary product (soup)

This example demonstrates the use of the cultured hydrolysate powder prepared as in example 2, as a flavour ingredient for a culinary application (mushroom soup). The soup has the following composition :

Ingredient	**[%]**
Onion powder	0.5
Cultured wheat gluten hydrolysate with enzymes (powder)	13.6

Modified starch	12.0
Skim milk powder	11.4
Wheat flour	13.0
Salt	2.4
Mushroom powder	6.5
Non-milk fat creamer	11.6
Maltodextrin	18.0
Fat	11

All the ingredients are mixed together in a conventional mixer. The soup is a dry culinary powder. 100g of powder are mixed with 800 ml of water and 200 ml partially skimmed milk and cooked for 5 minutes (simmering).

Example 6 : petfood composition

A mixture is prepared from 70 % of poultry carcass, pig lungs and beef liver (ground), 18 % of wheat flour, 8 % of water, and 0.2 % of liquid cultured hydrolysate as prepared in example 2, vitamins and inorganic salts.

This mixture is emulsified at 12°C and extruded in the form of a pudding, which is then cooked at a temperature of 90°C., It is cooled to 30°C and cut in chunks. 45 % of these chunks are mixed with 55 % of a sauce prepared from 98 % of water, 1 % of dye and 1 % of guar gum. Tinplate cans are filled and sterilized at 125°C for 40 min.

This petfood has a pleasant flavour as perceived by smelling the sample.

Claims

1. A process for the preparation of a cultured savoury base, which comprises hydrolysing a protein-containing material using a combination of at least one enzyme with at least one thermotolerant lactic acid bacteria strain selected for its ability to provide a glutaminase activity.

2. A process according to claim 1, wherein the hydrolysate is further processed downstream by means of thermal inactivation, filtration and/or centrifugation to produce a raw sauce.

3. A process according to claim 1 or 2, wherein the protein containing material is from plant or animal origin.

4. A process according to claim 3, wherein the protein containing material is wheat gluten, rice protein, soya, corn gluten, sunflower presscake, milk proteins and animal proteins.

5. A process according to one of claims 1 to 4, wherein the ratio enzyme : lactic acid bacteria strain is of about 1:1 to 4:1 based on % (w/w) addition of the ingredients.

6. A process according to one of claims 1 to 5, in which the enzyme is an exo- or endo- protease, deaminase, carbohydrase or amyloglucosidase.

7. A process according to claim 6, in which the enzyme is Flavorzyme®, Alcalase®, Dextrozyme® or AMG® or glutaminase C200®.

8. A process according to one of claims 1 to 7, in which the enzyme is used in an amount of at least 0.2 % (protease) or 0.05% (carbohydrase) by weight based on the total reaction.

9. A process according to one of claims 1 to 8, in which the thermotolerant lactic acid bacteria is selected from the group consisting of facultative heterofermentative Lactobacilli, *Lactobacillus rhamnosus*, *Lactobacillus delbrucki, Lactobacillus casei, Lactobacillus paracasei* or *Lactobacillus sp.*

10. A process according to one of claims 1 to 9, wherein the lactic acid bacteria is *Lactobacillus rhamnosus* (NCC 858) CNCM I-2433.

11. A process according to one of claims 1 to 10, in which the lactic acid bacteria is added in the form of a starter having a titer of about 1.10^8- 1.10^{11} cfu/g, in an amount of at least 0.1 to 0.5 % (w/w)

12. A process according to one of claims 1 to 11, wherein the hydrolysis is carried out for a time sufficient for the lactic bacteria strain to grow by 2-3 log, or to create 1-4% mono-sodium glutamate (MSG) in the hydrolysate, or to have a degree of hydrolysis (DH) of at least 20%.

13. A process according to one of claims 1 to 12, in which the hydrolysis is carried out at 43°C – 48°C, at pH 5- 6 for a time of at least 12 hours.

14. A process according to one of claims 1 to 13, wherein a slurry containing 15 to 30% of the protein containing material and 65 to 80% of water, is hydrolyzed using 1 to 4% of at least one enzyme such as Flavorzyme® combined with 0.5 to 2% of a lactic acid bacteria starter having a titer of about 1.10^5-1.10^6 cfu/g, in an amount of at least 0.1 to 0.5 % (w/w).

15. The use of a savory base prepared according to one of claims 1 to 14, as body giver in process flavour.

16. The use of a savory base prepared according to one of claims 1 to 14, for the preparation of seasonings, flavours and culinary or petfood products.

17. An isolated thermotolerant lactic acid bacteria strain selected for its ability to provide a glutaminase activity.

18. An isolated strain according to claim 17, which is selected from the group consisting of facultative heterofermentative Lactobacilli, *Lactobacillus delbrueckii, Lactobacillus casei, Lactobacillus paracasei* and *Lactobacillus rhamnosus* .

19. An isolated strain according to claim 17 or 18, which is *Lactobacillus rhamnosus* (NCC 858) CNCM I-2433.

20. The use of an isolated strain of lactic acid bacteria according one of claims 17 to 19, as process micro-organism for the preparation of seasonings, flavours and culinary or petfood products.

21. The use of an isolated strain according to claim 20, in combination with at least one enzyme.

22. The use according to claims 20 or 21, wherein the ratio enzyme : lactic acid bacteria strain is of about 1:1 to 4:1 based on % (w/w) addition of the ingredients.

INTERNATIONAL SEARCH REPORT

Int tional Application No
PCT/EP 01/03807

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A23L1/23 A23L1/238 A23J3/34 C12R1/225 C12R1/245

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A23L A23J C12R

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
WPI Data, PAJ, EPO-Internal, FSTA, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4 001 437 A (JAEGGI ET AL.) 4 January 1977 (1977-01-04) column 1, line 10 - line 24 column 1, line 65 -column 2, line 12 column 2, line 34 - line 41 column 3, line 6 - line 36; example 2 ---	1,3,4,6, 9,13,15, 16
X	DATABASE WPI Week 199532 Derwent Publications Ltd., London, GB; AN 1995-242691 XP002147980 & JP 07 147898 A (OGAWA KORYO KK), 13 June 1995 (1995-06-13) abstract --- -/--	1

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
31 July 2001	06/08/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Lepretre, F

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 01/03807

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE WPI Week 199314 Derwent Publications Ltd., London, GB; AN 1993-111797 XP002147981 & JP 05 049385 A (MEIJI MILK PROD.), 2 March 1993 (1993-03-02) abstract	1
X	US 3 852 479 A (YOKOTSUKA ET AL.) 3 December 1974 (1974-12-03) column 6, line 65 -column 7, line 19	17
A	EP 0 505 733 A (NESTLE) 30 September 1992 (1992-09-30) claims; example	1-16

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